

SEQUENCE LISTING

<110> Maxygen ApS
Maxygen Holdings Ltd.

<120> Single-Chain Polypeptides

<130> 0218us210

<150> US 60/245,727

<151> 2000-11-02

<160> 16

<170> PatentIn version 3.1

<210> 1

<211> 174

<212> PRT

<213> Homo sapiens

<400> 1

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
35 40 45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
50 55 60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
65 70 75 80

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
85 90 95

Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
100 105 110

Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
115 120 125

Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
130 135 140

Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
 145 150 155 160

Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
 165 170

<210> 2
 <211> 63
 <212> DNA
 <213> *Saccharomyces cerevisiae*

<400> 2
 atgaaattga aaactggttag atctgctggt ttgtcttctt tgtttgcttc tcaagttttg 60
 ggt 63

<210> 3
 <211> 126
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 3
 caaccaattg atgatactga atctcaaact acttctgtta atttgatggc tgatgatact 60
 gaatctgctt ttgctactca aactaattct ggtgggttgg atggtgttgg tttgatatcg 120
 atggcc 126

<210> 4
 <211> 522
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> DNA encoding G-CSF copy 1 in the single chain G-CSF dimer

<400> 4
 actccattgg gtccagcttc ttctttgcc caatcttttt tggtgaaatg tttggaacaa 60
 gttagaaaaa ttcaagggtga tgggtgctgct ttgcaagaaa aattgtgtgc tacttataaa 120
 ttgtgtcatc cagaagaatt gggtttgttg ggtcattctt tgggtattcc atgggctcca 180
 ttgtcttctt gtccatctca agctttgcaa ttggctggtt gtttgtctca attgcattct 240
 gggttggttt tgatcaagg tttgttgcaa gctttggaag gtatttctcc agaattgggt 300
 ccaactttgg atactttgca attggatggt gctgattttg ctactactat ttggcaacaa 360

atggaagaat tgggtatggc tccagctttg caaccaactc aaggtgctat gccagctttt 420
gcttctgctt ttcaaagaag agctggtggt gttttggttg cttctcattt gcaatctttt 480
ttggaagttt cttatagagt tttgagacat ttggctcaac ca 522

<210> 5
<211> 531
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding G-CSF copy 2 in the single chain G-CSF dimer

<400> 5
accctctgg gcccgccag cagtctgcct cagagttttt tactgaaatg cttagaacag 60
gtgcgtaaaa tccagggcga tggcgcggcc ctgcaggaaa aactgtgcgc gacctataaa 120
ctgtgccatc ctgaagaact ggtcctgtta ggccatagct taggcatccc gtgggcgcct 180
ctgagtagct gcccgagtca ggccctgcag ctggccggct gcctgagtca gttacatagt 240
ggcttatttt tatatcaggg cttactgcag gcgttagaag gcattagtcc ggaactgggc 300
ccgaccctgg ataccttaca gttagatgtc gcggattttg ccaccaccat ttggcagcag 360
atggaagaat taggcatggc gcctgcgtta cagcctaccc agggcgccat gcctgcgttt 420
gcgagtgcgt ttcagcgtcg cgccggcggc gtgttagtgg ccagccatct gcagagcttt 480
ctggaagtga gttatcgtgt gttacgccat ctggcccagc cttaatctag a 531

<210> 6
<211> 348
<212> PRT
<213> Artificial Sequence

<220>
<223> Single chain G-CSF dimer polypeptide

<400> 6

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
35 40 45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
50 55 60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
65 70 75 80

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
85 90 95

Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
100 105 110

Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
115 120 125

Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
130 135 140

Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
145 150 155 160

Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Thr Pro
165 170 175

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu
180 185 190

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys
195 200 205

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu
210 215 220

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser
225 230 235 240

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu
245 250 255

Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu
260 265 270

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala
 275 280 285

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu
 290 295 300

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg
 305 310 315 320

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu
 325 330 335

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
 340 345

Sequence

<210> 7
 <211> 90
 <212> DNA
 <213> Homo sapiens
 <400> 7
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 cacagtgcac tctggacagt gcaggaagcc 90

<210> 8
 <211> 522
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> DNA encoding single-chain G-CSF copy 1 (codon usage optimized for
 expression in CHO cells)

<400> 8
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 gttagaaaaa ttcaaggtga tgggtgctgct ttgcaagaaa aattgtgtgc tacttataaa 120
 ttgtgtcatc cagaagaatt ggttttgcttg ggtcattctt tgggtattcc atgggctcca 180
 ttgtcttctt gtccatctca agctttgcaa ttggctgggt gtttgtctca attgcattct 240
 ggtttgtttt tgtatcaagg tttgttgcaa gctttggaag gtatttctcc agaattgggt 300
 ccaactttgg atactttgca attggatggt gctgattttg ctactactat ttggcaacaa 360
 atggaagaat tgggtatggc tccagctttg caaccaactc aagggtgctat gccagctttt 420
 gcttctgctt ttcaaagaag agctgggtgg gttttgggtg cttctcattt gcaatctttt 480

ttggaagttt cttatagagt ttgagacat ttggtcaac ca

522

<210> 9
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 9

His His His His His His
1 5

<210> 10
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 10

Met Lys His His His His His His
1 5

<210> 11
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 11

Met Lys His His Ala His His Gln His His
1 5 10

<210> 12
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 12

Met Lys His Gln His Gln His Gln His Gln His Gln
1 5 10

<210> 13
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 13

Met Lys His Gln His Gln His Gln His Gln His Gln His Gln Gln
1 5 10 15

<210> 14
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 14

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

<210> 15
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 15

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> tag

<400> 16

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5